

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

Claim 1 (currently amended): A ligase-mediated method of recombination, comprising:
providing oligonucleotide fragments derived from each of at least two heterologous polynucleotide sequences of a polynucleotide bank;

hybridizing the fragments to an assembly matrix so that the hybridized fragments are oriented for ligation with each other; and

ligating the hybridized fragments having immediately adjacent ends with a ligase to form [[a]] random recombinant polynucleotide sequences. [[; and]]

~~selecting the recombinant polynucleotide sequence that exhibits advantageous characteristics compared to corresponding characteristics of one or more reference sequences.~~

Claims 2-49 (canceled).

Claim 50 (previously presented): The method of claim 1, further comprising at least one repetition of the providing step, the hybridizing step or the ligating step.

Claim 51 (previously presented): The method of claim 50, wherein the hybridizing step is repeated, before or after the ligating step, until the ends of more than half of the hybridized fragments are immediately adjacent to each other.

Claim 52 (previously presented): The method of claim 51, wherein, before a final ligating step, the ends of all of the hybridized fragments are immediately adjacent to each other.

Claim 53 (previously presented): The method of claim 1, wherein any polymerase extension performed during the hybridizing or ligating step, or between the hybridizing and ligating step, consists of gap filling between the hybridized fragments.

Claim 54 (previously presented): The method of claim 1, wherein the method is performed without a polymerase.

Claim 55 (previously presented): The method of claim 1, wherein the method is performed *in vitro*.

Claim 56 (previously presented): The method of claim 1, wherein, at the providing step, the fragments are cleavage fragments.

Claim 57 (previously presented): The method of claim 1, wherein, at the providing step, the fragments are random fragments.

Claim 58 (previously presented): The method of claim 1, wherein the method of recombination is a method of random recombination.

Claim 59 (previously presented): The method of claim 1, wherein the providing step comprises providing fragments that have been obtained in a manner such that degree of recombination desired and the position of the recombination points have been precisely controlled.

Claim 60 (previously presented): The method of claim 1, wherein the providing step comprises fragmenting the at least two heterologous polynucleotide sequences in a manner such that degree of recombination desired and the position of the recombination points have been precisely controlled.

Claim 61 (previously presented): The method of claim 1, wherein the at least two heterologous polynucleotide sequences differ from each other at more than one base position.

Claim 62 (previously presented): The method of claim 61, wherein the at least two heterologous polynucleotide sequences are derived from at least two distinct genes.

Claim 63 (previously presented): The method of claim 62, wherein the at least two heterologous polynucleotide sequences are derived from at least two distinct genes from at least two distinct gene families.

Claim 64 (previously presented): The method of claim 62, wherein the at least two heterologous polynucleotide sequences are derived from at least two distinct genes from at least two different species of organism.

Claim 65 (previously presented): The method of claim 1, wherein the at least two heterologous polynucleotide sequences are single-stranded.

Claim 66 (previously presented): The method of claim 1, wherein at least one assembly matrix is double-stranded and it is first denatured and then added in single-stranded form at the hybridizing step.

Claim 67 (previously presented): The method of claim 1, wherein at least one assembly matrix is single-stranded.

Claim 68 (previously presented): The method of claim 1, wherein the ligase is a thermostable ligase that is active at temperatures at or above 65°C.

Claim 69 (currently amended): The method of claim 1, wherein the polynucleotide bank comprises a variety of polynucleotide sequences obtained by mutagenesis or by combining genes of close or distinct families. ~~artificial polynucleotide sequences.~~

Claim 70 (previously presented): The method of claim 1, wherein, in addition to said fragments and assembly matrix, oligonucleotides of varying length, and single- or double-stranded, are added at the providing or hybridizing step.

Claim 71 (previously presented): The method of claim 1, wherein the polynucleotide bank comprises a restricted bank.

Claim 72 (previously presented): The method of claim 1, wherein the recombinant polynucleotide formed by the method is a non-naturally occurring polynucleotide.

Claim 73 (previously presented): The method of claim 1, further comprising cloning the recombinant polynucleotide sequence.

Claim 74 (previously presented): The method of claim 1, wherein a fragment from the providing step is used as the assembly matrix.

Claim 75 (previously presented): The method of claim 1, wherein the providing step comprises subjecting the at least two heterologous polynucleotide sequences to hydrolysis by the action of a plurality of different restriction enzymes or by the action of one or more restriction

enzymes having a large number of cutting sites on the at least two heterologous polynucleotide sequences.

Claim 76 (previously presented): The method of claim 75, wherein a fragment obtained at the providing step by a treatment with restriction enzymes is used as the assembly matrix.

Claim 77 (previously presented): The method of claim 1, wherein the providing step further comprises randomly fragmenting the at least two heterologous polynucleotide sequences by treating them with DNAase I.

Claim 78 (previously presented): The method of claim 77, wherein a fragment produced by the random fragmenting is used as the assembly matrix at the hybridizing step.

Claim 79 (previously presented): The method of claim 1, wherein the hybridizing and ligating steps are performed simultaneously.

Claim 80 (previously presented): The method of claim 1, wherein the at least two heterologous polynucleotide sequences are double-stranded and the providing step further comprises denaturing the fragments obtained at the providing step.

Claim 81 (canceled).

Claim 82 (previously presented): The method of claim 1, further comprising using the recombinant polynucleotide sequence as a source of fragments or as an assembly matrix during at least one repetition of the providing or hybridizing step.

Claim 83 (previously presented): The method of claim 1, further comprising separating the recombinant polynucleotide sequence formed at the ligating step from the assembly matrix.

Claim 84 (previously presented): The method of claim 83, wherein the recombinant polynucleotide sequence is separated from the assembly matrix using a marker present on the assembly matrix or on the recombinant polynucleotide sequence.

Claim 85 (previously presented): The method of claim 1, further comprising, before the selecting step, using polymerase extension to amplify the number of copies of the recombinant polynucleotide sequence.

Claim 86 (previously presented): The method of claim 1, wherein the selected recombinant polynucleotide sequence is used to form a new polynucleotide bank for a repeat of one or more of the providing step, the hybridizing step, the ligating step or the selecting step.

Claim 87 (previously presented): The method of claim 1, wherein the selection is performed by *in vitro* expression of the recombinant polynucleotide sequence.

Claim 88 (previously presented): The method of claim 1, further comprising using a degrading enzyme at the hybridizing or ligating step that specifically recognizes and degrades any nonhybridized ends of the fragments when said nonhybridized ends overlap hybridized fragments on the assembly matrix.

Claim 89 (previously presented): The method of claim 88, wherein the degrading enzyme is Flap endonuclease.

Claim 90 (previously amended): The method of claim 88, wherein the degrading enzyme and the ligase are equally thermostable at temperatures at or above 65°C.

Claim 91 (previously presented): The method of claim 88, wherein the degrading enzyme is an exonuclease that cleaves single-stranded nucleic acids.

Claim 92 (currently amended): A ligase-mediated method of recombination, comprising:
hybridizing oligonucleotide fragments derived from each of at least two heterologous polynucleotide sequences to an assembly matrix so that the hybridized fragments are oriented for ligation with each other; and

ligating the hybridized fragments having immediately adjacent ends with a ligase to form
[[a]] random recombinant polynucleotide sequences. [[; and]]

~~selecting the recombinant polynucleotide sequence that exhibits advantageous characteristics compared to corresponding characteristics of one or more reference sequences.~~

Claim 93 (new): The method of claim 1, wherein the recombinant polynucleotide sequences comprise at least two recombinant polynucleotide sequences.

Claim 94 (new): A ligase-mediated method of recombination, comprising:

providing oligonucleotide fragments derived from each of at least two heterologous polynucleotide sequences of a polynucleotide bank;

hybridizing the fragments to an assembly matrix so that the hybridized fragments are oriented for ligation with each other; and

ligating the hybridized fragments having immediately adjacent ends with a ligase to form at least two recombinant polynucleotide sequences.

Claim 95 (new): The method of claim 94, wherein the recombinant polynucleotide sequences are random.